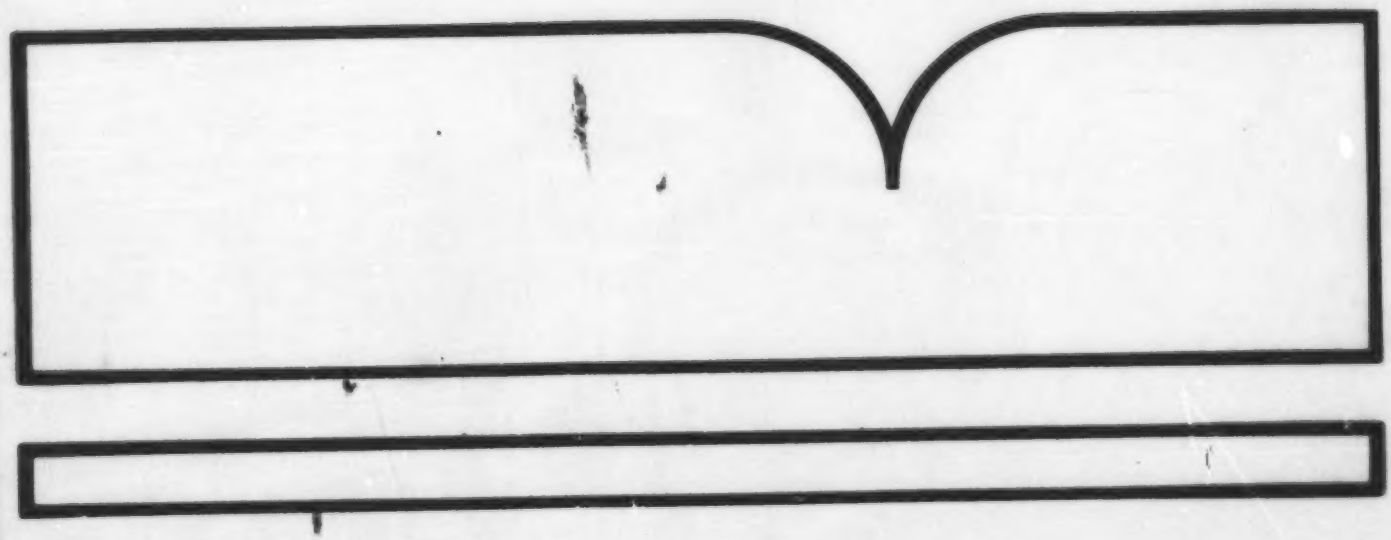
PB84-244185

Comparative Carcinogenic and Mutagenic Activity of Coal Tar and Petroleum Asphalt Paints Used in Potable Water Supply Systems

(U.S.) Health Effects Research Lab.
Cincinnati, OH

1984



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TECHNICAL REPORT DATA (Please read Instructions on the reverse before completin		
1. REPORT NO. EPA-600/J-84-088	2.	3. R PB84-244185
4. TITLE AND SUBTITLE Comparative Carcinogenic and Mutagenic Activity of Coal Tar and Petroleum Asphalt Paints Used in Potable Water Supply Systems (Journal Version)		5. REPORT DATE 1984 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) M. Robinson, R. J. Bull, J. Munch, & J. Meier		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS TOTB/TMD/HERL/USEPA 26 West St. Clair Street Cincinnati, OH 45268		10. PROGRAM ELEMENT NO. ABMCIA 11. CONTRACT/GRANT NO. In-house
12. SPONSORING AGENCY NAME AND ADDRESS Office of Research & Development HERL/USEPA Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED Final 14. SPONSORING AGENCY CODE EPA/600-11

15. SUPPLEMENTARY NOTES
Presented in part at 1982 Annual Meeting of Society of Toxicology, Boston, MA
Published: Journal of Applied Toxicology, Vol. 4, No. 1, 1984. pp. 49-56.

16. ABSTRACT

Coal tar and petroleum asphalt paints used as coatings for water pipes and storage tanks were tested in the Ames mutagenesis and the mouse skin carcinogenesis bioassays. To test the mutagenecity of the paints 6 doses ranging from 0.005 to 10 µl per plate were assayed. In the mouse skin bioassay doses of the coal tar paints ranging from 0.2 to 200 µl were administered topically to 30 SENCAR mice per group. These initiating doses were followed by applications of 1.0 µg 12-o-tetradecanoyl-phorbol-13-acetate (TPA) in 0.2 ml acetone topically 3 times weekly for 20 weeks. Petroleum asphalt paints were tested in groups of 40 animals at 200 and 600 µl doses.

All coal tar paints showed mutagenic activity after metabolic activation with S-9. None of the petroleum asphalt paints gave mutagenic responses. Both types of coatings resulted in positive responses in the initiation/promotion study. The coal tar paints gave rise to 1,000 to 1,800 times the tumor response observed with petroleum asphalt products. One coal tar product was positive when tested as a complete carcinogen in the mouse, whereas the asphalt paint was negative. The biological responses to the products were greater than expected from their polycyclic aromatic hydrocarbon (PAH) content. These findings suggest that the hazard posed by these coatings may not be fully explained by their PAH contents.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to Public	unclassified	9
	20. SECURITY CLASS (This page) unclassified	22. PRICE

REPRODUCED BY NATIONAL TECHNICAL INFORMATION SERVICE U.S. DEPARTMENT OF COMMERCE SPRINGFIELD, VA. 22161

EPA-600/J-84-088
JOURNAL ARTICLE

Comparative Carcinogenic and Mutagenic Activity of Coal Tar and Petroleum Asphalt Paints Used in Potable Water Supply Systems

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Key words: carcinogenesis; mutagenesis; drinking water; coal tar paint; petroleum asphalt paint; polycyclic aromatic hydrocarbons; mouse skin.

Coal tar and petroleum asphalt paints are among the products used as coatings for water pipes and storage tanks to retard corrosion. Formulations of these coatings were tested in the Ames mutagenesis and the mouse skin carcinogenesis bioassays. To test the mutagenicity of the paints, six doses ranging from 0.005 to 10 μl per plate were assayed. In the mouse skin bioassay, doses of the coal tar paints ranging from 0.2 to 200 μl were administered topically to 30 SENCAR mice per group. These initiating doses were followed by applications of 1.0 μg of 12-o-tetradecanoyl-phorbol-13-acetate (TPA) in 0.2 ml acetone topically, three times weekly for 20 weeks. Petroleum asphalt paints were tested in groups of 40 animals at 200 and 600 μl doses. All coal tar paints showed mutagenic activity after metabolic activation with S-9, with the highest response being in strains TA 98 and TA 100. None of the petroleum asphalt paints gave mutagenic responses. Both types of coatings resulted in positive responses in the initiation/promotion study. The coal tar paints gave rise to 1000–1800 times the tumor response observed with petroleum asphalt products. One coal tar product was positive when tested as a complete carcinogen in the mouse at 2 μl per application once weekly for 30 weeks, whereas the asphalt paint was negative at 100 times the dose. The biological responses to the products were greater than expected from their polycyclic aromatic hydrocarbon (PAH) content. These findings suggest that the hazard posed by these coatings may not be fully explained by their PAH contents.

INTRODUCTION

Coal-tar- and petroleum-asphalt-based coatings have been historically utilized in distribution systems for drinking water to prevent corrosion. The use of coal tar has been limited primarily to steel storage tanks and pipe. Petroleum-based linings have been used in cast-iron pipe that usually includes a prior coating of cement mortar.[1] Test panels and storage tanks coated with coal tar paints have been shown to contribute to the polycyclic aromatic hydrocarbon (PAH) content of water contacting the surfaces.[2] In contrast, the contribution of PAH by petroleum-asphalt-based coatings to water appears to be much lower, although still detectable.[3] These results are consistent with the higher PAH content of coal tar pitches versus those of petroleum asphalt pitches.[4]

Coal tar pitch has been clearly demonstrated to be carcinogenic in the mouse skin, whereas results with petroleum asphalt have been more variable.[4–6] However, it is not clear to what extent PAHs account for the carcinogenic activities of either coal tar or petroleum asphalt pitches. Early attempts to attribute the carcinogenic activity of tobacco smoke condensates to specific chemical substances revealed that PAHs accounted for only a small fraction of the total activity.[7] Similar results were obtained by Lijinsky *et al.* when trying to account for the carcinogenic activity of petroleum on the basis of its PAH content.[8] However, studies of petroleum asphalts were inconclusive because of a relatively low tumor yield.[4] The present study specifically examines coal tar and petroleum asphalt products used as coatings in potable water systems to determine whether their carcinogenic and mutagenic activities can be accounted for by their PAH content.

EXPERIMENTAL

Test materials

The coal-tar- and asphalt-based coatings were obtained from manufacturers of these products. Four different formulations of each type of paints were tested. The coal tar paints designated as E, F, G and H are formulated with varying percentages of coal tar pitch ranging from 37% (G) to 67% (E), and selected solvents (Table 1). All paints except E contain clay talc (magnesium silicate) filler. Coal tar G is not used in systems delivering potable water, but was tested for comparison with other paints. The asphalt-based paints designated as A, B, C and D have a primary component of an asphalt cutback. This 'cutback' is derived from a solid petroleum asphalt material cut back to 64% solid with mineral spirits. For the formulation variations, this 'cutback' is combined with additional mineral spirits and/or xylene. Total asphalt 'cutback' ranges from 89% (A) to 98% (B) (Table 1). TPA was obtained from Chemical Carcinogenesis, Eden Prairie, MN 55344.

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CCC-0260-437X/84/0004-0049$04.00

Table 1. Components of coal-tar- and petroleum-asphalt-based paints used in the Sencar mouse studies

Product	Components	Proportion (w/w; %)
Coal tar E	Coal tar pitch	67
	Xylene	33
Coal tar F	Coal tar pitch	47
	Clay talc[a] (filler)	16
	Solvents (toluene, perchloroethylene, xylene)	37
Coal tar G[b]	Coal tar pitch	37
	Clay talc (filler)	42
	Solvent (coal tar naphtha)	21
Coal tar H	Coal tar pitch	39
	Clay talc (filler)	30
	Solvents (toluene, perchloroethylene)	30
Asphalt A	Asphalt cutback[c]	89
	Xylene	1
	Additional mineral spirit	10
Asphalt B	Asphalt cutback	98
	Xylene	2
Asphalt C	Asphalt cutback	97
	Xylene	3
Asphalt D	Asphalt cutback	97
	Xylene	3

[a] Magnesium silicate.
[b] This formulation is not used in systems delivering potable water, but was tested for comparison with other paints.
[c] Solid petroleum asphalt material is cut back to 64% solid with mineral spirits prior to further formulation with mineral spirits and/or xylene.

Mutagenicity assay

The mutagenic potentials of the coal-tar- and asphalt-based paints were evaluated using the standard plate test method of the *Salmonella*/microsome assay, described by Ames *et al.*[9] *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 were employed. Assays were carried out both in the presence and absence of a rat liver microsomal activation system. The activation assays included 50 μl of S-9 fraction per plate, as well as the required cofactors. The procedures for Aroclor induction of the rat liver microsomal enzymes, and for preparation of the S-9 fraction and S-9 mix, were those used by Ames *et al.*[9] Assays were conducted at six dose levels of the paints: 0.005, 0.01, 0.1, 1.0, 5.0 and 10.0 μl per plate. These concentrations were attained by adding dilutions of the paints in DMSO at a constant volume of 50 μl per plate. Negative (solvent) and positive controls were assayed concurrently with each test sample. The positive controls in assays without S-9 activation were sodium azide for TA 1535 and TA 100, 2-nitrofluorene for TA 1538 and TA 98, and 9-aminoacridine for TA 1537. For activation assays, 2-aminoanthracene was used for all five strains. The criteria used for assessment of a positive response were the observations of twice the number of histidine-independent revertant colonies observed in the treated plates over the solvent control plates, and a dose-related increase in response. Mutagenic activities were quantitated by estimating the number of revertants per μl of sample from the linear portion (initial slope) of the dose-response curves.

Mouse skin bioassay

Female Sencar mice were obtained from Dr T. Slaga of Oak Ridge National Laboratories, Oak Ridge, TN. When the animals for tumor initiation/promotion experiments were 6 weeks of age, they were divided into groups of 30 animals (for coal tar) and 40 animals (for asphalt), and were treated with single doses of the paints ranging from 0.2 to 200 μl of the actual product applied to the shaved dorsal surface. Doses in excess of 200 μl were applied in multiple applications spaced 1 week apart. The paints were diluted with acetone to give a constant dosing volume of 0.2 ml. These treatments were followed 2 weeks later by topical application of TPA at 1.0 μg in 0.2 ml acetone per mouse, three times a week for 20 weeks. Benzo(*a*)pyrene [B(*a*)P; 10 μg per mouse] and 7,12-dimethylbenz(*a*)anthracene [DMBA; 2.56 μg per mouse] were used as positive controls. The B(*a*)P and DMBA were purified and provided by Dr F. B. Daniel of this laboratory.

To evaluate whether materials in these coatings were capable of acting as complete carcinogens, one product from each class was administered in a multiple-dose experiment. The dosing regimen consisted of 30 weekly applications of 2 μl (coal tar E) and 200 μl (petroleum asphalt D) per animal to the shaved dorsal surface of mice. Forty animals were utilized per experimental group. The weekly dosing schedule allowed the materials to wear off prior to subsequent applications. The animals received no tumor promoter. Tumor appearance and regression were charted from weekly observations for both the initiation/promotion study and the complete carcinogenicity study. To be included in the cumulative count, a tumor had to be present for three consecutive observations. All animals for each study were sacrificed at 52 weeks in the study by the Experimental Pathology Laboratories, Herndon, VA, who also performed the histopathological evaluations.

PAH analyses

The analyses of the coal tar paints were conducted by gas chromatography-mass spectrometry (GC-MS). Each of the samples was weighed and diluted to yield a solution of about 500 μg paint per ml of solution. A 2.0-μl sample, containing an internal standard of 1-chlorohexane (26 μg ml^{-1}), was injected on to a 30-m SE-54 quartz capillary column in the splitless mode. At the onset of the solvent peak, temperature programming was begun at a rate of 2 °C min^{-1} to a maximum temperature of 250 °C, which was maintained until the end of the run time. Data acquisition was terminated after 3900 mass spectra had been obtained. Data was collected similarly on standard solutions of 18 PAHs, and a library of reference spectra, retention times and response factors formed. The petroleum asphalt paints were analysed in the same manner as the coal tar except that a 60-m SE-30 capillary column was used. When it was found that the only PAH detected was naphthalene, a more concentrated sample was prepared by first preparing a 40 mg ml^{-1} solution of each paint in methylene chloride and then extracting with three, 4-ml portions of 3 N sodium hydroxide. With acidic interferences removed, 2.0 μl of the basic/neutral portion of the paint solution was then analysed. This procedure resulted in the analysis of 80 μg per assay (rather than the 1.0 μg per assay of the coal tar) and therefore lowered the detection limit. Even with the lowered detection limit, naphthalene re-

mained the only PAH detected above the quantification limit of 0.01 mg g^{-1}.

Each of the data files was searched by retention time for each of the PAHs in the library, the peak area determined from the single ion mass chromatogram for the compound, and the quantitation completed by comparing to the standard library value.

Chrysene and benz(*a*)anthracene, and benzo(*a*)- and benzo(*e*)-pyrene eluted as unresolved pairs, and estimates of their combined concentrations were made.

Table 2. Mutagenicity of petroleum asphalt and coal tar paints in the Ames test in the presence of metabolic activation

Coating type	Dose level (µl per plate)	Revertant colonies per plate[a]				
		TA 1535	TA 1537	TA 1538	TA 98	TA 100
Negative control	–	15±2	11±3	22±4	38±3	157±33
Positive control (2-amino-anthracene)	2.5	494±115	353±129	1837±383	1612±300	2106±136
Coal tar E	0.005	20	14	23	43	128
	0.010	15	15	30	61	170
	0.100	19	19	38	87	253
	1.000	14	34	63	112	348
	5.000	25	40	83	152	354
	10.000	17	34	84	162	355
Coal tar F	0.005	14	36	51	161	280
	0.010	16	51	78	226	351
	0.100	21	101	204	576	545
	1.000	22	85	568	622	478
	5.000	3	39	89	208	130
	10.000	0	9	16	92	13
Coal tar G	0.005	16	38	54	146	248
	0.010	17	62	94	268	356
	0.100	14	103	313	704	561
	1.000	25	205	980	1370	676
	5.000	13	175	974	1244	757
	10.000	9	119	678	1540	266
Coal tar H	0.005	23	43	45	128	288
	0.010	23	54	84	176	332
	0.100	35	137	183	387	500
	1.000	29	179	610	913	476
	5.000	29	75	427	755	312
	10.000	3	39	238	445	254
Asphalt A	0.005	19	17	26	41	118
	0.010	17	12	28	53	126
	0.100	16	14	19	38	163
	1.000	12	14	28	42	146
	5.000	17	14	22	53	159
	10.000	27	15	27	49	158
Asphalt B	0.005	12	12	15	35	134
	0.010	22	12	23	36	135
	0.100	19	7	25	39	121
	1.000	18	10	20	42	156
	5.000	18	16	28	40	146
	10.000	16	12	23	41	120
Asphalt C	0.005	18	14	22	49	211
	0.010	16	15	13	37	195
	0.100	19	13	22	47	195
	1.000	15	19	25	47	173
	5.000	16	17	18	53	198
	10.000	15	16	26	55	215
Asphalt D	0.005	16	6	19	44	217
	0.010	15	10	23	46	246
	0.100	16	11	21	39	226
	1.000	14	7	23	54	231
	5.000	15	10	24	47	231
	10.000	12	9	17	43	226

[a] All assays were carried out with metabolic activation (+ S-9). Values are averages from duplicate plates for assays with test compound and mean values ± SD from 10 plates for control assays.

RESULTS

Salmonella/microsome assay

Neither the coal-tar- nor the petroleum-asphalt-based paints showed evidence of mutagenic activity in the absence of microsomal (S-9) activation (data not shown). Furthermore, none of the asphalt based paints gave mutagenic responses in the presence of S-9 activation (Table 2). On the other hand, all of the coal-tar-based paints showed mutagenic activity after metabolic activation with S-9. Positive responses were observed with *Salmonella* strains TA1537, TA1538, TA98 and TA100, but not with TA1535 (Table 2). The highest mutagenic responses of the coal tar paints were observed with either strain TA98 or TA100, depending on the product. Toxicity to the bacterial tester strains was apparent for all the coal tar paints, except coal tar E, at the higher dose level, as indicated by a decline in mutagenic response to below the background level. Toxicity was not evident for the asphalt products.

Mouse skin assay

Figure 1 shows the dose response of the Sencar mouse to topical doses of B(*a*)P ranging from 0.3 to 30 μg per mouse expressed both as tumors per animal and proportion of

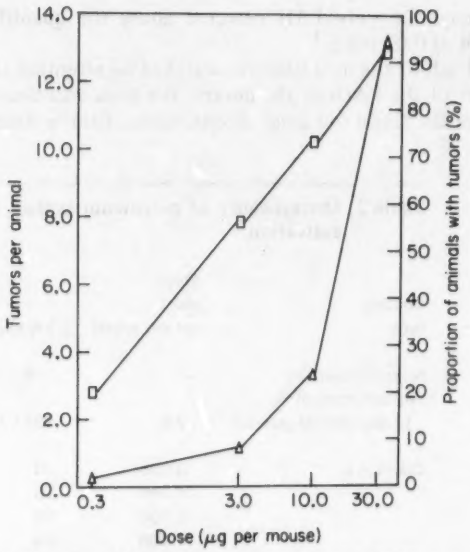

Figure 1. Tumor yield in Sencar mice treated with B(*a*)P: △, tumors per animal; □, proportion of animals with tumor (%). Doses were administered topically in a single application and followed in 2 weeks with the tumor promoter TPA being applied at a dose level of 1.0 μg in 0.2 ml acetone three times weekly for 20 weeks.

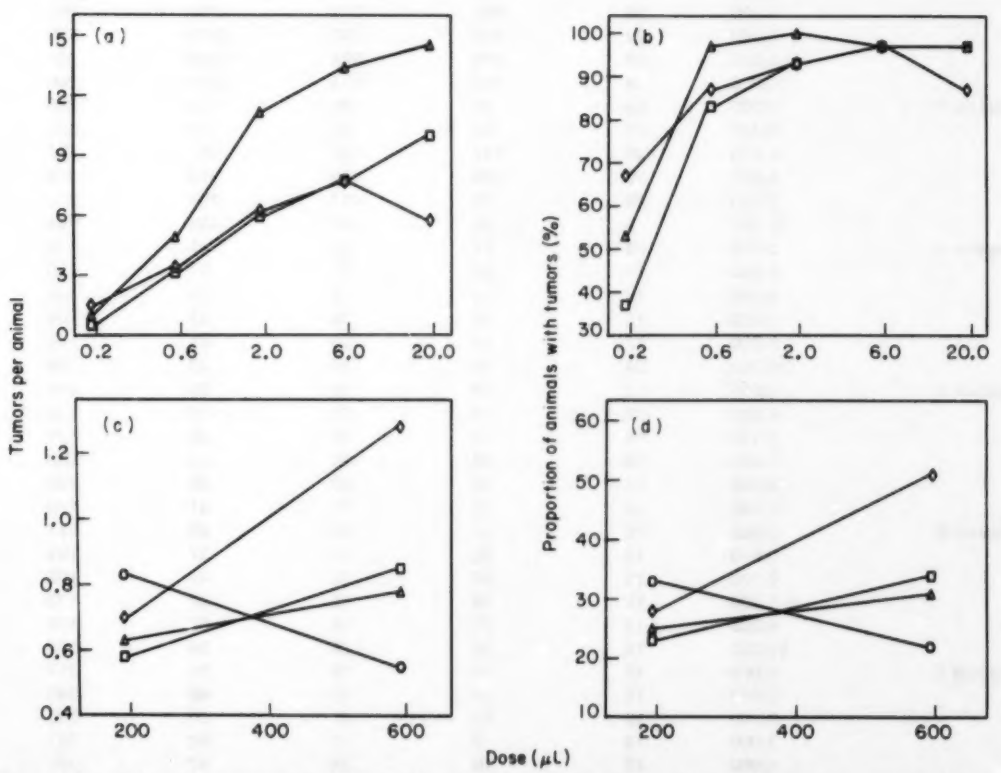

Figure 2. Tumor yield resulting from treatment of Sencar mice with (a and b) coal tar and (c and d) petroleum asphalt paints used in water supply systems. There were 30 animals in each group of animals receiving coal tar paint (△, paint E; □, paint F; ◇, paint G) and 40 per group in those treated with petroleum asphalt paints (△, paint A; □, paint B; ◇, paint C; ○, paint D). Two weeks following the indicated tumor-initiating doses, the tumor promoter TPA was applied at 1.0 μg in 0.2 ml acetone per application, three times weekly for 20 weeks. Data shown is after 52 weeks on study.

animals with tumors. It is important to recognize that the Sencar strain has been selected for its sensitivity to PAH and substantially greater tumor yields are observed for equivalent doses of B(*a*)P relative to other strains of mouse commonly used in initiation/promotion experiments in the skin.[13]

As shown in Fig. 2, both coal tar- and petroleum asphalt-based paints initiated tumor development in the mouse skin. The activity associated with the coal tar paints was much greater than that observed with the petroleum asphalt paints. A clear dose response was seen with the coal tar products, with sample E displaying the maximum tumor yield. While sample G produced significantly greater tumor yields at low doses (0.2 μl per mouse), the tumor yield rose to a lesser extent with increasing dose and actually decreased at doses above 6.0 μl. Petroleum asphalt paints gave rise to increases in tumor yield and significantly increased the proportion of animals bearing tumors (multiple-cell chi-square analysis) relative to animals treated with mineral spirits alone ($P<0.05$) at a dose of 200 μl per mouse. Formulations A, B and C displayed a dose-related increase at 600 μl per mouse. However, paint D displayed a decreased tumor yield at the higher dose, despite the fact that it gave rise to the highest response at the lower dose.

Histopathological findings on tumors examined in the initiation/promotion assays of coal tar and petroleum asphalt paints are presented in Table 3. The differentiation between squamous cell carcinoma and squamous cell papilloma was based on hyperactivity of basal layer, penetration of the basement membrane with invasion into the underlying dermis and subscutis, cellular pleomorphism, individual keratinized cells, increased mitotic activity and, in some instances, by metastasis to regional lymph node or the lung. Sarcomas of the dermis were also present in one or two mice in each group of the coal tar study, except for E (0.2 μl), F (6.0 and 0.2 μl) and G (0.2 μl). These sarcomas were generally found only in animals also bearing carcinomas. Despite the fact that a number of animals were lost to histopathological examination through autolysis and/or cannibalism following early death, it is clear that treatment with coal tar paints gave rise to substantial numbers of malignant tumors. Generally, the frequency of squamous cell carcinoma was dose related, except for an unusually high yield of carcinomas at the 0.6 μl dose of coal tar E. The yield of squamous cell carcinomas in animals treated with petroleum-asphalt-based paints were considerably less than that observed with coal tar paints, consistent with their lower overall tumor yield. As a group, however, the yield of carcinomas was significantly greater than control.

In the experiment to test the complete carcinogenicity of these products, only the coal tar product proved to be positive (Table 4). The 2.0 μl of coal tar E, applied weekly

Table 3. Classification of histologically-examined tumors from animals treated with coal tar and petroleum asphalt paints

Treatment	Dose (μl)[a]	*n*		Number of animals with:		
		Initiated	Examined	Squamous cell tumor[b]	Squamous cell papilloma[c]	Squamous cell carcinoma[c]
Coal tar E	0.2	30	23	12	10	3
	0.6	30	26	24	13	20
	2.0	30	18	14	10	10
	6.0	30	20	19	14	11
	20.0	30	14	14	10	7
Coal tar F	0.2	30	25	5	5	1
	0.6	30	27	17	13	7
	2.0	30	23	20	14	14
	6.0	30	21	16	11	13
	20.0	30	22	21	13	15
Coal tar G[d]	0.2	30	26	14	13	4
	0.6	30	21	13	9	9
	2.0	30	19	15	10	10
	6.0	30	21	21	17	11
	20.0	30	26	15	6	12
Acetone	0.2 ml	30	23	4	4	0
Benzo(*a*)pyrene	10.0 μg	30	27	15	11	9
Asphalt A	200	40	36	6	4	2
Asphalt B	200	40	31	5	5	0
Asphalt C	200	40	31	8	4	5
Asphalt D[c]	200	40	33	9	9	6
DMBA	2.56 μg	20	8	8	3	6

[a] Doses were delivered topically in a single application per animal. Two weeks following the initiating dose, tumor promotion was achieved by applying 12-otetradecanoyl-phorbol-13-acetate at 1.0 μg in 0.2 ml acetone per application, three times weekly for 20 weeks.

[b] One to two mice in all coal tar groups except E (0.2), F (6 and 0.2) and G (0.2) also had subcutaneous sarcomas. Most of these mice also had squamous cell carcinomas. The asphalt D group also had one animal with a fibrosarcoma and one with a basal cell carcinoma.

[c] Total number of animals having squamous cell papillomas and/or carcinomas does not agree with number of animals with squamous cell tumor, because some animals had both types.

[d] This formulation is not used in systems delivering potable water, but was tested for comparison with other paints.

Table 4. Complete carcinogenicity of coal-tar- and asphalt-based paints in the Sencar mouse

Product	Dose[a]	Papillomas		Carcinomas	
		Animals with tumors (%)	Total tumors	Animals with tumors (%)	Total tumors
Coal tar E	2.0 μl	33/40 (83)	171	4/40 (10)	5
Asphalt D	200.0 μl	1/40 (3)	1	0	0
Mineral spirits	200.0 μl	3/40 (8)	3	0	0
Benzo(*a*)pyrene	100.0 μg	26/40 (90)	210	7/40 (18)	17

[a] Dose administered once weekly for 30 weeks. Coal tar E diluted with mineral spirits to give 2.0 μl of material delivered in 0.2 ml per application.

for 30 weeks, resulted in 83% (33/40) of the animals bearing a total of 171 tumors. This was comparable to the response observed when 100 μg of B(*a*)P was applied weekly for 30 weeks (26/40 animals bearing 210 tumors). Ten percent of the mice receiving coal tar E had carcinomas (four animals had five carcinomas). Only 3% (1/40) of the animals that received asphalt D had tumors, and there were no carcinomas present. The vehicle control resulted in 8% (3/40) of the mice with tumors, again without evidence of malignancy.

PAH analyses

Chemical analyses of the four coal tar paints indicated high levels of PAH, whereas concentrations of PAH in the two asphaltic-based paints examined were very low (Table 5). Five compounds, identified primarily in the coal tar paints, that have been shown to be biologically active in both the mouse skin bioassay and in the Ames mutagenicity test are chrysene, benzo(*a*)anthracene, B(*a*)P, benzo(*e*)pyrene and phenanthrene. The combined concentrations of these five compounds amount to 44% of the total PAH in coal tar E, 42% in coal tar F and 33% in coal tar G. None of these PAHs were found in the asphalt samples except for a trace of phenanthrene in both asphalts A and D.

Comparison of biological activity with PAH contact

In Table 6, the tumor yield in the mouse skin and the degree of mutagenic activity observed per μl of coal tar paint is compared with the activity which could be expected based on the concentration of PAHs identified in the paints that are known to be carcinogenic and/or mutagenic. Due to incomplete data on these compounds in the Sencar mouse, we have utilized the data of DiGiovanni *et al.*[13] and Scribner[14] in the CD-1 mouse. It was assumed that the ranking of the potencies of these chemicals would be the same in the Sencar and the CD-1 mouse, and the calculations were adjusted according to the

Table 5. Concentrations of polyaromatic hydrocarbons (PAH) in coal tar and petroleum asphalt paints

Polyaromatic hydrocarbon	Concentration of coal tar (mg g^{-1})				Concentration of asphalt (mg g^{-1})	
	E	F	G	H	A	D
Naphthalene	<1[a]	1.0	13.0	<1	0.2	0.3
Biphenyl	ND[b]	<1	<1	ND	<<0.01	<0.01
Acenaphthalene	ND	ND	ND	ND	<0.01	<0.01
Acenaphthene	2.4	1.9	1.0	3.2	ND	ND
Fluorene	1.4	1.5	1.0	1.2	<0.01	<0.01
9-H-Fluorene	ND	ND	ND	ND	<0.01	ND
Phenanthrene	15.7	13.6	7.8	6.9	<0.01	<0.01
Anthracene	3.4	3.5	2.0	1.5	<0.01	ND
Acridine	ND	<1	ND	<1	<0.01	<0.01
2-Methylphenanthrene	2.7	<1	1.1	<1	<0.01	<0.01
2-Methylanthracene	<1	<1	<1	<1	ND	<0.01
Fluoranthene	5.6	14.8	11.9	5.3	ND	ND
Pyrene	13.6	11.8	10.2	5.4	ND	ND
1-Methylpyrene	1.2	<1	<1	ND	ND	ND
Chrysene + benzo(*a*)anthracene	6.6	10.4	10.7	5.9	ND	ND
Benzo(*a*)pyrene + benzo(*e*)pyrene	3.4	1.9	3.0	3.4	ND	ND

[a] Present at less than the quantitation limit of 1 mg g^{-1} for coal tar and 0.01 mg g^{-1} for petroleum asphalt.
[b] ND = not detected. May be present below the quantitation limit of 1 mg g^{-1} for coal tar and 0.01 mg g^{-1} for petroleum asphalt.

Table 6. Comparison of actual carcinogenic and mutagenic activity with theoretical activity based on PAH contents of coal tar paints

Paint	Average tumor yield per μl product	Theoretical tumor yield per μl product based on PAH contents[a]	Net revertants TA 100 per μl of product	Theoretical revertants TA 100 per μl product based on PAH contents[a]
E	7.0	1.17	4136	1777
F	3.7	1.12	24 200	2327
G	4.1	1.32	24 900	2853
H[b]	–	–	13 450	2298

[a] Calculated on basis of the activity attributable to the measured amounts and relative mutagenicity or carcinogenicity of benzo(*a*)pyrene, benzo(*e*)pyrene, chrysene, benzo(*a*)anthracene and phenanthrene in the CD-1 mouse (mutagenicity, ref. 12; carcinogenicity, refs. 13 and 14). Unresolved pairs assumed to be present in the ratio quoted in ref. 4, i.e. B(*a*)P/B(*e*)P = 0.6/0.4 and chrysene/benzo(*a*)anthracene = 0.45/0.55.

[b] Tumor yield not determined for this sample, since a multiple-dose experiment was not conducted. A single-dose experiment did indicate activity that approximated other coal tar paints.

documented differences in sensitivity of these strains to PAHs.[13] The data of McCann *et al.*[12] was utilized to make a similar calculation for *Salmonella* strain TA 100. In both cases, the ratio between unresolved pairs in the PAH analysis were estimated on the basis of the data of Wallcave *et al.*[4] (i.e. B(*a*)P/B(*e*)P = 60/40; chrysene/benzo(*a*)anthracene = 45/55).

Differences between the observed biological response and that expected based upon PAH content were substantial. The theoretical tumor yield was fairly uniform for the coal tar products, ranging from 1.12 to 1.32 tumors per μl. On the other hand, the measured response varied by almost a factor of two (3.7 for coal tar F to 7.0 for coal tar E). In all three cases, the actual tumor yield substantially exceeded the theoretical tumor yield. Even more substantial discrepancies exist between actual and theoretical levels of mutagenic activity. Calculated levels of activity that could be attributed to measured PAH content ranged from 1777 to 2853 revertants per μl, while the actual ranged from 4136 to as high as 24 900 net revertants per μl. The descrepancy between actual and theoretical mutagenic activity ranged from three- to more than ten-fold.

DISCUSSION

It is clear from the present work that both coal tar and petroleum asphalt paints contain chemicals capable of initiating cancer. The carcinogenic and mutagenic activities of coal tar enamels confirms previous studies of the carcinogenic effects of coal tar pitch.[4,5] Absence of mutagenic activity and considerably less potent tumor-initiating activity with petroleum asphalt-based enamels are consistent with the much less potent carcinogenic activity that has been associated with petroleum asphalt pitch.[4-6] Nevertheless, it is clear that petroleum asphalt paints are capable of initiating tumors in the mouse skin and that a significant number of these tumors are carcinomas.

All of the coal tar paints exhibited mutagenic activity in the *Salmonella*/microsome assay. In every case, activation by microsomal enzymes was required before activity could be detected. The coal tar paints were active with all three of the *Salmonella* strains that detect frameshift mutagens (i.e. TA 1537, TA 1538 and TA 98), and also with TA 100, which can detect mutagens that induce both frameshift and base-pair substitution mutations. On the other hand, none of the coal tar paints reverted strain TA 1535, which carries the same histidine mutation as TA 100, but which is specific for base-pair substitution mutations. These findings suggested that the compounds responsible for the mutagenic activity of these paints are all frameshift mutagens.

Within the limits of the doses applied, only coal tar enamel was capable of acting as a whole carcinogen. Application of 2.0 μl for 30 weeks resulted in 83% of the mice developing papillomas with the average tumor yield of 4.3 tumors per animal. Of those animals bearing tumors, 81% developed carcinomas within 1 year. In contrast, petroleum asphalt formulation D did not give rise to either papillomas or carcinomas at 100 times the dose. This, of course, does not rule out the possibility that petroleum asphalt paints would not give rise to tumors at higher doses (i.e. 200 μl per week must be considered a relatively modest dose for complete carcinogenesis bioassay).

There were few correlations between the composition of these paints and their biological activity. It is reasonably clear that the differences in PAH content can account for a substantial portion of the difference in carcinogenic and mutagenic responses between coal-tar- and petroleum-asphalt-based formulations. However, the extent to which PAH content accounts for the differences in these responses to paints within the coal tar group is not at all clear. Neither tumor-initiating or mutagenic activity was related to total PAH content. Although the ranking of coal tar paints by mutagenic potency was consistent with their content of mutagenic PAHs, the response to coal tar E was disproportionately less than would have been predicted. To complicate the analysis, this same paint was clearly the most potent product in the mouse skin as a tumor initiator. Even though this product contained the greatest proportion of coal tar pitch, its content of tumorogenic PAHs was less than for product G.

Despite the lack of clear cut correlations within these data, it is clear that in no case does the content of biologically active PAH account for the activity observed in either the mouse skin or the *Salmonella*/microsome assay. This type of discrepancy has been observed in other similar products.[4,7,8,10] The difference between observed and expected activity with these products may be due to unidentified components, synergism between components, or

some combination of these two explanations. Synergistic effects have been observed for B(*a*)P and B(*e*)P. Rao *et al.*[11] reported an enhancement of B(*a*)P-induced mutagenesis in the Ames test by B(*e*)P of up to 3.6-fold, depending on the relative amounts tested. In either case, it is clear that the overall hazard associated with these products cannot be dependably estimated based upon the activity of individually identified components.

Some attention must be called to the remarkable carcinogenic and mutagenic potency of the coal tar enamels which are applied to drinking water storage and distribution systems. A single dose of 0.2 μl of these paints is sufficient to initiate tumors in 35–65% of the animals. Repeated application of 2.0 μl on a weekly basis was capable of producing malignant tumors in the absence of secondary treatments (e.g. TPA). The hazard these paints present to consumers of drinking water can only be thoroughly evaluated with knowledge of the extent to which carcinogenic and mutagenic chemicals leach into potable water. Addressing that problem is beyond the scope of the present study. In the past, this aspect of the problem has been considered primarily in terms of the PAH concentrations that the coating contributes to the drinking water.[2] The results presented here suggest that this may not be an adequate representation of the hazard posed by these coatings.

Acknowledgements

We would like to express our thanks to David Cmehil for his technical assistance in completing the in-house mouse skin bioassays and to Pat Underwood for her assistance in the preparation of this manuscript. The mutagenesis testing was performed under contract by Litton-Bionetics, Kensington, Maryland.

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Received 11 April 1983; accepted (revised) 19 July 1983